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Glutathione (GSH) and superoxide dismutase (SOD) levels among junior high school students induced by indoor particulate matter 2.5 ($PM_{2.5}$) and nitrogen dioxide (NO_2) exposure

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Significance for public health

Indoor air pollution has globally known as one of public health problem and the risk factor of acute respiratory infection in young children. The exposure to indoor particulate matter 2.5 (PM2.5) and nitrogen dioxide (NO_2) at house or school can be a potential risk to children's health. This study aimed to examine the association between indoor PM2.5 and NO_2 with oxidative stress markers in junior high school students and help affirm association towards student's health and to design strategic control efforts.

Abstract

Background: Indoor air pollution has globally known as the risk factor of acute respiratory infection in young children. The exposure to indoor particulate matter 2.5 ($PM_{2.5}$) and nitrogen dioxide (NO_2) at house or school can be a potential risk to children's health. This study aimed to examine the association between indoor $PM_{2.5}$ and NO_2 with oxidative stress markers in junior high school students.

Design and Method: This study was conducted using a cross sectional study with 75 students collected randomly from four junior high schools in Jakarta. $PM_{2.5}$ and NO_2 were measured in classrooms and school yards. The schools were categorized based on the exposure level of $PM_{2.5}$ and NO_2 in classrooms. Superoxide dismutase (SOD) and reduced glutathione (GSH) were examined from the blood sample. All students were interviewed with questionnaires to determine upper respiratory tract infection, smoking family members, mosquito repellent usage, and dietary supplement consumption.

Results: Mean concentration of indoor $PM_{2.5}$ and NO_2 were 0.125±0.036 mg m^{-3} and 36.37±22.33 µg m^{-3}, respectively. The schools which located near to highway showed lower $PM_{2.5}$ and higher NO_2 level indicated the emission of traffic activity. Mean activity of SOD was 96.36±50.94 U mL^{-1} and mean concentration of GSH was of 0.62±0.09 µg mL^{-1}. Most of the students reported upper respiratory tract infection history, smoking family member, use mosquito repellent at home, and do not consume dietary supplement.

Conclusion: The level of oxidative stress markers and the exposure categories of classroom $PM_{2.5}$ and NO_2 was not significantly different, however there were significant correlation with cigarette smoke and mosquito repellent at home. Nevertheless, the exposure of indoor $PM_{2.5}$ and NO_2 increased the risk of the exposure to cigarette smoke and mosquito repellent at home. Further study on the air pollution at school and home is needed to affirm association towards student's health and to design strategic control efforts.

Keywords: School; particulate matter; indoor air quality; oxidative stress; antioxidant

Introduction

Indoor air pollution is one of the major health risk factors responsible for nearly 1.6 million excess deaths annually and about 3% of the global burden of disease[1]. Outdoor air pollution strongly influences indoor air quality especially due to human activities such as traffic and industrial activities[2,3]. Previous study reported that, based on the guideline of World Health Organization, most of the municipality of Jakarta province had poor air quality from 2011 to 2017 and was reported to be associated with respiratory infection among population in Jakarta[4,5]. Traffic activity is one of the major sources of air pollutants such as particulate matters ($PM_{2.5}$) and nitrogen dioxide (NO_2) in Jakarta. Particulate matter with diameter of less than 2.5 μm or $PM_{2.5}$ is a major component of air pollution that can be inhaled up to systemic circulation [6,7]. Meanwhile, NO_2 gas is mainly emitted from traffic activity that involves fuel burning and directly combines with the oxygen in the atmosphere [8,9].

Vigorous development activities in Jakarta have increased air pollution source points such as highways or toll roads into adjacent school buildings, impacted to increase the exposure risk of $PM_{2.5}$ and NO_2 to students in school environment[2,10]. School-age children are vulnerable to environmental exposure due to their immature immune and respiratory system. During school time, this group spends most of their time six to eight hours at school [11,12]. Several studies reported that high exposure of $PM_{2.5}$ and NO_2 in classroom were associated with respiratory problems in children such as asthma, decreased of lung function, and respiratory tract and lung inflammation [11,13–15]. Several studies have reported that airborne air pollutants can induce oxidative stress resulted to increase the risk of respiratory problems[8,16]. Inhaled air pollutants such as $PM_{2.5}$ and NO_2 initiate reactive oxygen species (ROS) production that induce inflammation response in lung resulting oxidative stress indicated by depletion of specific antioxidants or elevation of antioxidant activity such as superoxide dismutase (SOD)[8,16]. Superoxide dismutase (SOD) plays as an enzymatic antioxidant to catalyze the dismutation of superoxide anion radical to molecular oxygen and harmless hydrogen peroxide which is then to be scavenged by glutathione (GSH) through enzymatic reactions. GSH is the most important hydrophilic antioxidant that protects cells against free radicals. Disorder of antioxidants level has been implicated in the etiology or development of various diseases [6,17]. However, study on oxidative stress effects to the inflammation of respiratory tract on school-age children in Jakarta has not well understood, particularly the production of SOD and GSH. Thus, a study on antioxidant levels of SOD and GSH was suggested to early determination on the health risk of $PM_{2.5}$ and NO_2 exposure towards students in Jakarta.

Design and Method

This study was conducted from December 2010 to January 2020 in Jakarta Special Region, Indonesia, using a cross-sectional design. Four junior high schools were selected randomly from four municipalities in Jakarta. Parental and student informed consents were distributed randomly to 200 first grade of junior high school students living around five kilometers from the school. Students who were willing and permitted to participate from their parents underwent examination by medical doctors to fulfill the inclusion and exclusion criteria. This research excluded students with the history of chronic respiratory disease and who were experiencing symptoms of health problems on the day of blood sampling.

Data collection

Interview was conducted to collect demographic and health information. We conducted structural interview to 75 students using standard questionnaires adapted from a set of validated questionnaires by International Study of Asthma and Allergies in childhood (ISAAC) and food frequency questionnaires. The questionnaire was used to determine history of upper respiratory tract infection, tobacco smoke exposure at home, mosquito repellent usage, and dietary supplement intake. The same questionnaire was used in previous school studies in Depok, West Java [10,18]. The selected students were interviewed by researchers based on the questionnaire.

Indoor air samples

We collected ambient air samples at each school to measure $PM_{2.5}$, NO_2, temperature, and humidity. Samples were collected both indoor (one classroom) and outdoor (one school yard) during school time. $PM_{2.5}$ concentration was measured using DustTrak II Aerosol Monitor which monitored continuously for 8 hours (8 am to 4 pm) in one school day. The inlet flow was positioned at one meter above the ground level and distant to walls and doors to represent the actual children's respiration zone [19,20]. NO_2 was monitored for one hour (8 am to 9 am) in one school day using Impinger according to the standardized Griess-Saltzman method by National Standardization Agency of Indonesia to measure absorbed NO_2 [21]. In addition, we also measured the classroom's physical conditions (i.e., temperature and humidity) and observed the classroom condition. Each school was assessed in different week due to school permit, academic activities, and availability of the instruments.

Superoxide dismutase (SOD) and glutathione (GSH) level in blood

Blood samples were collected from the students who have agreed to the term and conditions of the study. Around 5 ml blood samples were collected using sterile syringe by experienced paramedic. Blood samples were then centrifuged to separate plasma and stored into vial. Samples were then kept in refrigerator at 4°C. SOD enzyme concentration was assayed by using RanSOD Kit and read by RX-Monza. This measurement aimed to get the dismutation of the toxic superoxide anion to hydrogen peroxide and oxygen. Xanthine and Xanthine oxidase were used to generate superoxide anion radicals. It reacts with 2-(4-iodoponhenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T) to create a red formazan dye. SOD activity was measured by inhibition degree of the reaction[22].

GSH was measured using spectrophotometric method. GSH was oxidized by 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) resulting in the formation of oxidized glutathione (GSSG) and 5-thio-2-nitrobenzoic acid (TNB). GSSG then was reduced to GSH by glutathione reductase (GR) using reducing equivalent provided by NADPH. The rate of TNB formation was proportional to the sum of GSH and GSSG presented in the sample and was determined by measuring the formation of TNB at 412 nm.[23] The analysis was done in Biochemistry Laboratory, Faculty of Medicine, Universitas Indonesia.

Statistical analysis

All data in this study were analyzed using statistical analysis software package. The Skewness-Kurtosis method was used to test data normality. Students' demographic data, nutrition status, upper respiratory tract infection history, home tobacco smoke exposure, mosquito repellent usage, and dietary supplement intake were analyzed descriptively. Schools were categorized into highly exposed and low exposed categories based on mean levels of indoor $PM_{2.5}$ and NO_2. To evaluate association between the exposure and oxidative stress marker, the mean values of SOD and GSH between exposure categories were compared using *t*-test and Mann Whitney test, with p-value <0.05 considered statistically significant. The mean of SOD and GSH between the categories of students' demographic, nutrition status, upper respiratory tract infection history, home tobacco smoke exposure, mosquito repellent usage, and dietary supplement intake were also compared using t-test and Mann Whitney test.

Results

Subjects and location characterization

All corresponding schools in this study open in five school days per week at 6:30 am to 2:15 pm. Besides that, extracurricular activities were also performed until around 4:00 pm. Two of the four schools (school A and D) were located next to the highway, while school B was located about 100 m from the main highway, and school C was in a residential area (Table 1). All classrooms were floored without carpets and were naturally ventilated with additional fans. All classrooms were furnished with wooden desks, chairs, and whiteboards. All school yards have cement or asphalt flooring.

A total of 75 students from the four schools voluntary participated as subject and met the inclusion and exclusion requirements for the study (Table 2). The mean age of the subject was 13.06-year-old which most subjects were female students. Most of the students had smoking family member, used mosquito repellent, and did not consume dietary supplement at least once per week in last one month. Our results found that 76% students have upper respiratory tract history in last six months.

Exposure of $PM_{2.5}$ and NO_2

The mean $PM_{2.5}$ concentrations in four schools were 0.125 mg/m^3 in classroom and 0.090 mg/m^3 in school yard (Table 3). School C (residential area) had the highest $PM_{2.5}$ concentration for both inside and outside of the classroom. All schools have higher $PM_{2.5}$ concentrations inside the classroom compared to the yard. Based on the mean $PM_{2.5}$ concentrations, school B and C were categorized as high $PM_{2.5}$/ low NO_2.

The mean concentrations of NO_2 were 36.37 mg/m^3 and 19.24 mg/m^3 in in classroom and school yard, respectively. School A has the highest NO_2 concentration (62.54 mg/m^3) in classroom. All schools, except for School D, showed higher NO_2 concentrations in classroom compared to school yard. Meanwhile, NO_2 concentration in School D showed no difference concentration for both inside classroom and in school yard. School D had higher NO_2 concentration than the other schools. Based on the mean concentrations, school A and C were highly exposed to NO_2 which then categorized as high NO_2/ low $PM_{2.5.}$ $PM_{2.5}$ and NO_2 measurements were carried out in the rainy season with clear or cloudy weather patterns in the morning until noon and cloudy or rainy in the afternoon. All classrooms have higher humidity levels and lower temperatures compared to school yard. $PM_{2.5}$ and NO_2 concentrations were also found to be higher in the classroom than in school yard.

Oxidative stress marker

The mean SOD activity from all samples was 96.36 U mL^{-1} and the mean concentration of plasma GSH was 0.62 µg mL^{-1} (Table 4). Based on our statistical analysis, there was no significant difference of GSH and SOD levels between high PM2.5 schools and high NO2 schools. w. However, schools with high NO_2 exposure in the classroom had lower levels of GSH and higher SOD activity (Table 5). Meanwhile, mean SOD activity and GSH concentration between sex (p=0.889; 0.819), nutritional status (p=0.368; 0.612), history of upper respiratory tract infection (p=300; 0.190), and dietary supplement consumption (p=0.213; 0.617), were not statistically different. However, there was a statistically significant difference of mean SOD activity (p=0.001) and mean GSH concentration (p=0.036) between cigarette exposure and non-cigarette exposure at home. The usage of mosquito repellent resulted in a significant difference in GSH concentration (p=0.035).

Discussion

Airborne particulate matter and nitrogen dioxide

The concentration of $PM_{2.5}$ in four schools was similar to a previous study conducted in schools in Depok, Indonesia and several studies in India[18,24,25]. Several other studies conducted in Germany, Italy, Portugal, and Malaysia reported that $PM_{2.5}$ concentrations that exceeded WHO guideline values, but still lower than our current study[11,12,26,27]. According to the previous studies, high $PM_{2.5}$ levels in the classroom might be caused by poor ventilation, poor classroom hygiene, occupant activities, and other outdoor sources[11,26,28]. However, we did not make further observation for these factors in this study. In contrast to the studies in Germany and Malaysia, this study found that schools located in suburban settlements (far from the highway or industry) had the highest $PM_{2.5}$ concentrations[26,29]. another study conducted in kindergarten in Portugal reported a similar results with this study[11,12]. Higher $PM_{2.5}$ concentrations in a residential area might be caused by public activities such as biomass burning and cooking, open yards or land without cement layering, and surrounding activities such as traffic and industry[30,31].

The $PM_{2.5}$ concentration in this study showed that rainy season did not influence the air quality in the school environment. Studies in tropical and subtropical countries reported a decreasing pattern of outdoor particulate levels which related to the increase of humidity and rainfall during rainy season. Indoor particulate level can increase during the rainy season due to student's activity in the classroom and poor ventilation openings [11,25,26,28,30].

The mean NO_2 concentration in four schools did not exceed the threshold values set by the Indonesian government for one hour (400 µg m^{-3}, 1 hour) and WHO (200 µg m^{-3}, 1 hour). A similar study by Pratama *et al*. (2018) on indoor air pollution in Jakarta reported lower indoor and outdoor NO_2 than our results. Other studies conducted in the United States and Brazil measured NO_2in the school environment reported there was association of NO_2 in air and respiratory disorders [15,32,33]. Whereas studies in India and Australia reported higher NO_2 and correlated with respiratory disorders, even though they did not exceeded the WHO threshold value [25,34].

The existence of NO_2 in ambient air was specifically related to outdoor air pollutants sources such as traffic activities, thus related to the increase of NO_2 concentration in classroom and yard in school A and D. This results indicated that outdoor air pollutants affected the indoor air quality in the classroom[25,33]. Beside that, the lowest NO_2 concentration was detected in school C (suburban settlement) indicated that buildings which located far from highway or main road received less NO_2 pollution. Previous studies also suggested that NO_2 levels was relatively lower in the rainy season and increase during the dry season [25].

SOD and GSH levels

At the time of data collection, subjects had just enrolled in the school for six months, thus these results represent the exposure of the last six months before data collection. The median SOD activity in this study was higher than a study conducted among healthy, asthma, and down syndrome children, but was similar to a study among workers in the ready-mix concrete factory exposed to $PM_{2.5}$[35,36]. The production of SOD activity occurred in response to oxidative stress due to an increase in superoxide reactive species. This can be mediated by air pollutants exposure, such as particulates or nitrogen dioxide [22,37]. Our results found that the students with high NO_2 exposure had slightly higher SOD activity and slightly lower GSH concentration than the students with low NO_2 exposure. A previous study by Bernard et al. (2016) also reported lower GSH concentration and no significant correlation was observed between GSH in adults and NO_2 (higher than 40 µg

m^{-3}) [38]. However, the mean plasma GSH concentration of all students in this study was lower than some other studies conducted in healthy children [39,40]. The increased in reactive species levels in a certain concentration and time will pose to the depletion of GSH concentration resulted in the decrease of cell capability against oxidative stress. Depleted GSH has been reported to be associated with health risk of respiratory system disorder such as chronic pulmonary disease, acute respiratory distress syndrome, neonatal lung damage and asthma [8,17,40,41]. In this study, the students with reported upper respiratory tract infection showed lower GSH level but there was no significant correlation between GSH and upper respiratory tract infection.

Despite f the exposure to air pollutants in schools, the level of antioxidants might be associated with the exposure to air pollutants at home such as mosquito repellent or cigarette smoke[42–44]. In this study, students who were exposed to mosquito repellent at home showed significantly lower GSH levels. Previous studies on pyrethroid-based mosquito repellent and antioxidants in mice showed the decrease of GSH level in the brain[42,45]. Mosquito repellent contains pyrethroid compounds that produce reactive species during the metabolism process and induce oxidative stress in a certain time and concentration[42,46,47]. Meanwhile, the mean GSH concentration and SOD activity showed significant different between students exposed to and not exposed to cigarette smoke at home. Cigarette smoke typically contains more reactive species which easily induce oxidative stress easily resulted in the decrease of antioxidant levels in the human body [38,48]. However, in this study, SOD activity and GSH concentrations were higher in students exposed to cigarette smoke. Research by Yokus *et al*. (2015) on oxidative stress among active and passive smokers suggested that SOD activity will increase as an adaptive response to the oxidative load of cigarette smoke and clean up excess superoxide reactive species [49]. Adaptive response of GSH will also increase gradually to achieve their normal level in a certain period of time [50]. However, the adaptive response can be disrupted or decrease with increase in age[49,50].

Limitations

This study did not record the number of daily cigarettes and periods of smoking among family members so the frequency of exposure could not be further explained specifically.

Conclusion

The concentration of $PM_{2.5}$ and NO_2 showed higher level in the schools located near to highway or main road. The relation between $PM_{2.5}$ and NO_2 exposure with antioxidant level showed no significant correlation. However, there was a significant difference between SOD and GSH concentration with the students who were exposed to cigarette smoke and mosquito repellent. The upper respiratory tract infection history in students suggested that students had been exposed to poor air quality in both school and house. Further study should be improved to measure other airborne pollutants which related to the increase of oxidative stress by determining other potential factors.

Data availability

The data used to support the study are available from the corresponding author upon request.

Ethical clearance

This study has been reviewed and passed the ethical clearance from Faculty of Public Health Universitas Indonesia with the reference number of 665/UN2.F10.PPM.00.02/2019.

Conflict of interest

Authors declared no conflict of interest in this study.

Authors' contribution

The study presented here was carried out in collaboration between all authors. All authors prepared the study design and protocols. BW, HK, JJ, and SP1 interpreted and drafted the manuscript. BW, JJ, SP1, SP2, NF, and FF conducted data collection and analysis, RN, SP1 and GP edited and translated the manuscript. All authors have read and agreed to the manuscript submission.

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Table 1. School characteristic.

School	Location	Sampling sites	Classroom shape	Classroom area (m^2)	Number of students/class	Environment condition
A	Pasar Baru, Central Jakarta	1st floor classroom (1) & school yard (1)	Rectangle	62.01	32	City center, business and office areas, next to main highway, near the intersection of red lights and the train station.
B	Lubang Buaya, East Jakarta	3rd floor classroom (1) & school yard (1)	Rectangle	64.8	36	Suburban, office and business areas, 100 m from the main highway and red light intersections
C	Cipedak, South Jakarta	3rd floor classroom (1) & school yard (1)	Rectangle	64.74	36	Suburban, residential areas, and next to golf courses.
D	Kebon Jeruk, West Jakarta	3rd floor classroom (1) & school yard (1)	Rectangle	56.15	36	Suburban, business and residential areas, next to main highway, and the intersection of red lights.

Table 2. Subject characteristic.

Characteristic		Description
Age (year)		
Mean±SD		13.06±0.48[a)]
Sex (n, %)		
Female	58	77.33
Male	17	22.67
Nutrition Status (n, %)		
Thin	3	4.00
Normal	48	64.00
Fat	24	32.00
Upper respiratory tract infection history (last 6 months) (n, %)		
Yes	57	76.00
No	18	24.00
Smoking family member (n, %)		
Yes	43	57.33
No	32	42.67
Dietary supplement consumption (at least once per week in last month) (n, %)		
Yes	32	42.67
No	43	57.33
Mosquito repellent usage (n, %)		
Yes	42	56.00
No	33	44.00
SOD (U mL^{-1})		
Mean±SD		96.36±50.94
Median		84.97[b)]
GSH (µg mL^{-1})		
Mean±SD		0.62±0.09[a)]
Median		0.63

a) normal distribution, b) skewed distribution

Table 3. Level of pollutants in schools

Parameter	Mean ± SD									
	Classroom				School Yard				Classroom (n=4)	School Yard (n=4)
	School A	School B	School C	School D	School A	School B	School C	School D		
$PM_{2.5}$ ($mg\ m^{-3}$)	0.095	0.128	0.175	0.103	0.092	0.109	0.095	0.065	0.125±0.036	0.090±0.018
NO_2 ($\mu g\ m^{-3}$)	62.54	24.5	12.37	46.05	23.72	4.33	2.87	46.05	36.37±22.33	19.24±20.24
Humidity (%)	73.5	71.43	77.03	76.77	69.53	56.83	68.77	72.73	75.24±2.65	68.73±7.21
Temperature (°C)	31.57	31.00	30.37	30.63	32.23	37.10	34.87	32.17	30.74±0.57	33.36±2.62

Table 4. Level of oxidative stress markers in students

Antioxidant	School A n = 21	School B n = 23	School C n = 16	School D n = 15	All Schools n = 75
SOD (U/ mL)					
Mean ± SD	84.31±23.11	76.81±47.39	139.82±71.32[a)]	96.86±31.17[a)]	96.36±50.94
Median	83.86[b)]	62.27[b)]	129.21	101.88	84.97[b)]
CI 95%	73.79-94.83	56.32-97.31	101.82-177.82	79.59-114.13	48.64-108.08
Min –Max	45.58-124.09	39.62-262.44	39.62-273.45	43.01-133.78	39.62-273.45
GSH (µg/ mL)					
Mean ± SD	0.52±0.08[a)]	0.61±0.05[a)]	0.66±0.0.06[a)]	0.72±0.05[a)]	0.62±0.09[a)]
Median	0.51	0.61	0.66	0.72	0.63
CI 95%	0.48-0.56	0.59-0.63	0.63-0.70	0.69-0.74	0.60-0.64
Min –Max	0.33-0.68	0.51-0.73	0.57-0.83	0.51-0.73	0.33-0.83

Note: a) normal distribution, b) skewed distribution

Table 5. Oxidative stress marker association to various variables

Variable	Antioxidants level					
	SOD (U mL^{-1})			GSH (µg mL^{-1})		
	n=75	Mean ±SD / Median (IQR)	p-value	n= 5	Mean ±SD / Median (IQR)	p-value
$PM_{2.5}$ & NO_2 exposure						
High $PM_{2.5}$/Low NO_2	39	83.03(69.49)	0.714	39	0.63±0.06	0.197
Low $PM_{2.5}$/High NO_2	36	89.54(51.42)		36	0.60±0.12	
Sex						
Female	58	83.44(60.10)	0.889	58	0.62±0.09	0.819
Male	17	93.92(60.17)		17	0.62±0.10	
Nutrition status (BMI age)						
Normal	27	83.03(53.35)	0.368	27	0.63±0.09	0.662
Abnormal (Thin & Fat)	48	88.23(63.68)		48	0.61±0.10	
Upper respiratory tract infection history (last 6 months)						
Yes	57	80.29(59.09)	0.300	57	0.61±0.10	0.190
No	18	104.25(64.57)		18	0.65±0.09	
Smoking family member						
Yes	43	110.71(62.96)	**0.001**	43	0.64±0.09	**0.036**
No	32	68.99(36.44)		32	0.59±0.10	
Dietary supplement consumption (at least once per week last month)						
Yes	32	92.93(43.94)	0.213	32	0.60±0.10	0.617
No	43	98.93(55.97)		43	0.63±0.09	
Mosquito repellent usage						
Yes	42	89.82(53.44)	0.601	42	0.60±0.10	**0.035**
No	33	84.87(73.19)		33	0.64±0.08	